Journal of Forestry Research (2009) 20(1):27–30
DOI 10.1007/s11676-009-0005-3

RESEARCH PAPER

Variations in seed germination of *Hippophae salicifolia* with different pre-soaking treatments

S. Airi, I.D. Bhatt *, A. Bhatt, R.S. Rawal, U. Dhar

G.B. Pant Institute of Himalayan Environment and Development, Kosi- Katarmal, Almora - 263 643 (UK), India

Abstract: Mature seeds of *H. salicifolia,* collected from five provenances (i.e. Hanuman Chatti, Helang, Lata, Rambara and Janggal Chatti) in west Himalaya, India, were treated with stratification (at 4ºC for 15, 30 and 60 days) and in different concentrations of GA_3 (5, 10, 20 mM), KNO_3 (50, 100, 200 mM) and Thiourea (50, 100, 200 mM) solution to determine the variations in seed germination. Results reveal that the germination rates of seeds from different provenances under different pre-sowing treatments are significantly increased compared to those in control (24%–30%). The seeds treated with Thiourea (100 mM) have highest germination rate (76%–83% for different seed sources), followed by those (63%–71% for different seed sources) pretreated with stratification (4^{o}C, 30 days). GA_3 treatment significantly shortens the mean germination time (MGT) and improves seed germination percentage. Considering the practical applicability and cost effectiveness, thiourea (100 mM) and stratification (at 4^{o}C) treatments for seed germination are recommended for mass multiplication through seeds of *H. salicifolia* in village/forest nurseries of the west Himalaya, India.

Keywords: Seed germination percentage; *Hippophae salicifolia*; presoaking treatment; stratification; Thiourea

Introduction

Hippophae salicifolia D. Don (Vernacular- Chuk, Tarwa) is a deciduous tree species restricted to the Himalayan region, between 1500–3500 m a.s.l. (Hooker 1990; Gaur 1999). The species bear red and yellow berries, which are edible, a rich source of vitamins, and used in preparations of various products including local beverages (Gaur 1999). The flavanoids, fatty acids and other bio-active compounds of *H. salicifolia* berries might be capable of reducing the incidence of cancer (Mathews 1994). The species is also considered as fine vegetation in improving soil fertility and restoring degraded sites in high hills (Gamble 1972; Huxley 1992).

H. salicifolia generally propagates by root suckers. However, extensive collection and short growing season can hamper mass multiplication of the species. The natural regeneration of the species through seed is also very scanty (Sankhyan et al. 2005). Sankhyan et al. (2005) studied the seed germination of *H. salicifolia* collected from single population. However, to achieve higher productivity and select suitable genotypes for future breeding programmes, seed source testing is important (Mamo et al. 2005). Therefore, in the present study we designed several presoaking treatments for seeds of *H. salicifolia* collected from different provenances, aimed at achieving a high germination percentage and short mean germination time and assessing the variation in seed germination among populations.

Methods

Mature seeds of *H. salicifolia* were collected during first and second week of November (2004) from five provenances (Hanuman Chatti, Helang, Lata, Rambara and Janggal Chatti) in west Himalaya, India (Table 1). Provenances were selected on the basis of (1) distant localities at diverse altitude (1715–2965 m a.s.l.), (2) association with diverse forest communities, and (3) substantially large population with mature individuals. Five apparently healthy mature trees located inside the population in each provenance were identified for collection of seeds. Marginal plants were avoided. All the seeds collected were ensured to be apparently at same stage of development and the infected seeds were discarded. Immediately after collection, seeds were cleaned manually, sunned to be dried for seven days, and stored in brown paper bags at (25±2)ºC until the start of experiments.

Seed viability was tested using 1% (w/v) solution of Tetrazolium chloride TTC (2, 3, 5 triphenyl tetrazolium chloride, $C_{19}H_{15}N_4Cl$, Sigma) with pH adjusted at 6.0 (Hendry and Grime 1993). Fifty dry seeds from each provenance were soaked in 1% aqueous solution of TTC for 24 h and kept in dark at room temperature. Seeds were bisected longitudinally and examined under

Received: 2008-05-29; Accepted: 2008-07-24

The online version is available at http://www.springerlink.com
Biography: S. Airi (1966), male, Masters in Botany, Technician –B in G.B. Pant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora - 263 643 (UK), India.
(Email: airisubodh@rediffmail.com)
*Corresponding author: Bhatt I.D. (Email: idbhatt@gbpihed.nic.in; id_bhatt@yahoo.com)
Responsible editor: Zhu Hong

a microscope to determine viability percentage. Seeds with a red-stained embryo were considered to be viable.

Various pre-sowing experiments were performed to determine the effects of different treatments on seed germination percentage and mean germination time (MGT). A set of seeds without pre-sowing treatments was considered as control. Every treatment with three replicates of 60 seeds was arranged from individual provenance.

Table 1. Site characteristics of identified various provenances of *H. salicifolia*

Various provenances	Altitude (m, asl)	Aspect	Seed weight (g) n=10	Latitude	Longitude	Seed moisture content (%)	Dominant associated tree species
Helang (P_1)	1715	NE	0.14 (±0.010)	30º34′	79º34′	27	*Alnus nepalensis*
Lata (P_2)	2310	NW	0.15 (±0.009)	30º29′	79º44′	31	*Alnus nepalensis*, *Picea smithiana*
Hanuman Chatti (P_3)	2600	NE	0.16 (±0.014)	30º43′	79º17′	34	*Alnus nepalensis, Abies pindrow, Taxus baccata*
Rambara (P_4)	2820	NW	0.13 (±0.004)	30º44′	79º03′	29	*Betula utilis, Abies pindrow*
Jangal Chatti (P_5)	2965	NE	0.14 (±0.005)	30º35′	79º30′	33	*Alnus nepalensis*

Notes: Values in parenthesis represent standard deviation

Selected seeds were surface-sterilized by dipping in 0.5% aqueous solution of mercuric chloride for 2 min to remove bacterial and fungal contamination and then rinsed thoroughly (four times) in distilled water. The sterilized seeds were soaked for 24 h in different concentration of GA_3 (5, 10, 20 mM), KNO_3 (50, 100, 200mM) and Thiourea (50, 100, 200mM) solutions. For the cold stratification, seeds were kept at 4 ºC for 15, 30 and 60 days and then placed at (25±2) ºC. A set of untreated seeds were used for comparing the results. Treated seeds were washed thoroughly with distilled water and placed in Petri plates (95 mm ×17mm) containing moistened filter paper (Whattman No.1). Petri plates were kept at 25±2ºC in growth chamber and moistened as needed with distilled water. The date of first germination was recorded for each treatment. The seed with emergence of radicle (>5 mm long) was considered as seed germinated. The number of seeds infected by fungi and bacteria was recorded each day and removed from Petri plates. The whole experiment was monitored up to 90 days. MGT was calculated as:

$$MGT = \sum(nd) / N \quad (1)$$

where, n is the number of seed germination after each incubation period in days d, and N is the total number of seeds germination at the end of test (Hartmann and Kester 1989).

Statistical analysis

All the experiments were conducted in a completely randomized block design. Analysis of variance (ANOVA) was performed with the help of SYSTAT (Wilkinson 1986). Data on germination percentage were subjected to arcsine transformation before being considered for ANOVA. Fishers Least Significant Difference (F-LSD) was estimated separately for the comparison of treatment and provenance means (Snedecor et al. 1967).

Results

The viability pattern of the seeds stained by Tetrazolium test shows that there is no significant difference in viability of the seeds from different provenances (Fig.1). Invariably high (84%–92%) viability of *H. salicifolia* seeds indicates that seeds would largely be capable of germination under favorable conditions and that failure of germination implies dormancy of seeds.

The seed germination percentage (Fig. 1 and Table 2) for untreated seeds (control set) is low (i.e. 24.7% to 30.7%). The percentage of seed germination, however, is significantly higher ($p<0.05$) from two provenances (P_2 and P_5) compared to others. Under various pre-sowing experiments, seeds from different provenances do not exhibit uniformity in responses (Table 2, 3).

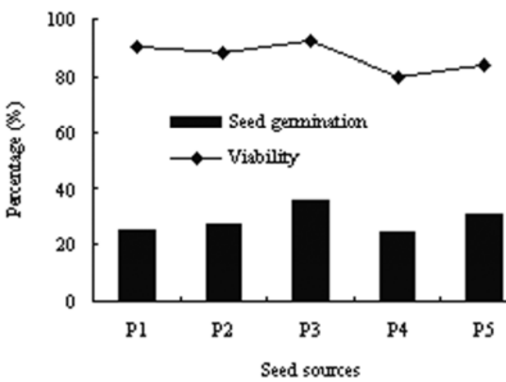

Fig. 1 Percentage of seed viability and seed germination in control set of *Hippophae salicifoliaa*

P1----Hanuman Chatti, P2----Helang, P3----Lata, P4----Rambara, P5----Janggal Chatti

Under all the pre-sowing treatments, cold stratification and Thiourea treatments show maximum improvement in mean percentage of seed germination over control (Table 2). In case of cold stratification, seed germination percentage is maximum (58%–71.3%) under 30-day stratification. Considering the responses across provenances, significantly ($p<0.05$) more germination of seeds is revealed from P_5 (71.3%) and significantly less (58.0%) from P_4 provenance. In case of Thiourea, seed germination percentage increases with increase in Thiourea concentration up to 100mM. Further increase in concentration of Thiourea significantly ($p<0.05$) reduces the germination percentage in most cases. The highest mean seed germination (83.3%- Helang (P_1)) under 100-mM thiourea treatment is significantly ($p<0.05$) better than that of P_4 and P_5 provenances. As compared to cold

stratification and thiourea, seeds soaked in GA_3 (different Conc.) resulted in lesser improvement in germination. Responses under KNO_3 treatment are comparable with GA_3 treated seeds.

Table 2. Seed germination percentage of *Hippophae salicifolia* from different provenances and under different pretreatments

Treatments	Seed germination rate (%)					LSD (p<0.05)	F-ratio
	Helang (P_1)	Lata (P_2)	Hanuman Chatti (P_3)	Rambara (P_4)	Jangal Chatti (P_5)		
Control	27.34	35.34	25.33	24.66	30.66	4.50	9.46**
Stratification							
15 days	44.00	62.00	54.67	50.66	55.33	4.60	20.52**
30 days	63.34	65.00	66.00	58.00	71.33	2.16	10.30**
60 days	55.34	61.67	58.67	56.00	60.66	3.85	5.18*
Thiourea							
50mM	72.00	68.66	72.66	68.66	73.00	3.54	3.68*
100mM	83.33	81.34	80.00	77.33	76.66	5.54	2.49^{ns}
200mM	62.66	62.66	78.00	74.44	70.00	3.95	30.24**
GA_3							
5mM	38.66	40.66	33.33	42.66	43.33	4.12	9.43**
10mM	38.00	36.00	26.00	56.33	46.66	4.79	57.98**
20mM	38.66	34.66	33.33	45.33	49.33	6.28	11.93**
KNO_3							
50mM	47.34	46.00	44.00	29.33	51.33	2.82	88.50**
100mM	66.00	64.66	64.67	31.33	68.66	5.71	73.89**
200mM	54.00	53.34	49.33	32.33	56.00	5.63	28.96**
LSD (p<0.05)	7.20	4.35	4.53	2.39	4.82		
F-ratio	41.61**	99.82**	152.52**	332.64**	67.75**		

Notes:* p<0.05, **p<0.01, ns : non significant.

The best responses were observed for KNO_3 (100 mM). Further increase in concentration of KNO_3 (>100 mM) lowered the mean seed germination significantly in most cases (except P_4 provenance).

The mean seed germination time (MGT) for untreated seeds (control set T_1) across provenances varied from 15.2d to 19.5d. Compared to control, all the treatments (except GA_3 (10 mM)), caused increase in mean germination time (Table 3).

Table 3. Effect of best pre-sowing treatment on mean germination time (MGT) of *Hippophae salicifolia* seeds

Seed source	Mean germination time of seed (d)					Mean
	T_1	T_2	T_3	T_4	T_5	
P_1	15.18	21.84	14.70	20.95	16.80	17.89
P_2	18.27	23.81	13.86	22.49	17.47	19.18
P_3	15.63	22.33	14.46	18.10	16.31	17.35
P_4	18.96	24.67	13.36	18.28	17.67	18.59
P_5	19.49	25.31	10.99	20.01	19.32	18.98
Mean	17.50	23.58	13.47	19.97	17.51	

Notes: LSD (p<0.05) for treatment and seed source mean 2.09; 2.89.T_1 – Control; T_2- Cold Stratification (30 days); T_3-GA_3 (10 mM); T_4-KnO_3 (100 mM) and T_5- Thiourea (100 mM).

Discussion

Variability in seed germination ability for several species has been reported among seed sources in different regions (Tewari and Dhar 1996; Siril et al. 1998). Causes of such variations are attributed to (1) genetic characters of different populations/plants (Bewley and Black 1994) or (2) impact of mother plant environment (Anderson and Milberg 1998), or (3) wider habitat conditions (Friis 1992), or (4) diverse altitudinal gradients (Rawat et al. 2007). These factors could be considered as important factors affecting germination characteristics of *H. salicifolia*.

The high seed viability percentage (84%–92%) and poor germination (24.7%–30.7%) of untreated seeds (Fig. 1) would suggest prevalence of dormancy among seeds of *H. salicifolia*. As such, the members of family Elaegnaceae mostly have the fruits with stony endocarp (Baskin et al. 1998) and embryo having deep physiological dormancy, which require cold stratification in long periods to overcome dormancy (Nikolaeva 1969). The Improved germination responses under cold stratification for *H. salicifolia* in our study are agreed with these reports.

Our study result indicates that seed germination of *H. salicifolia* can be improved significantly through various pre-sowing treatments. Thiourea and cold stratification treatments are proven to be superior over GA_3 and KNO_3 treatments for seed germination of *H. salicifolia*. Improved seed germination under thiourea treatment is in agreement with reports of many other species (Chaudhary et al. 1996; Mclntyre et al. 1996; Pandy et al. 2000). Likewise, improvement of germination in stratification at 4ºC is in agreement with the report of Sankhyan et al. (2005) who reported 53% germination in the cold water (2 d). However, stratification at longer duration (30 d) had seed germination of 58%–71.33%. It is found that stratification at 2–5ºC for different time duration is effective in number of other species for seed germination (Stokes 1969, Khan et al. 1969) including *H. rham-*

noides (Pearson et al. 1962).

The improved seed germination rates under Thiourea and cold stratification treatments reveal that both the pretreatments are quite simple and inexpensive. These two pre-sowing treatments can be widely used by the village/forest nurseries in the Himalayan region and can be recommended for large scale plantlet production of the species.

Acknowledgements

We thank the Director, GBPIHED for support and encouragement. Thanks are due to Dr. S.S. Samant, and Mr. K. Gaira for their help and support.

References

Anderson L, Milberg P. 1998. Variation in seed dormancy among mother plants, populations and year of seed collection. *Seed Science Research*, **8**: 29−38.

Baskin CC, Baskin JM 1998. *Seed*: ecology, biogeography and evolution of dormancy and germination. California (USA): Academic Press, p 668.

Bewley JD, Black M. 1994. *Seed physiology of development and germination*. New York: Plennum Press, p 221.

Chaudhary DK, Kaul BL, Khan S. 1996. Breaking seed dormancy of *Podophyllum hexandrum* Royle ex Camb (syn. *P. emodi* Wall. Ex Honigberger). *Journal of Non Timber Forest Products*, **3**: 10−12.

Friis I. 1992. *Forests and Forest Trees of Northeast Tropical Africa*: their natural habitats and distribution patterns in Ethiopia, Djibouti and Somalia. London: Royal Botanic Gardens, p396.

Gamble JS. 1972. *A Manual of Indian Timbers*. 2nd ed. Dehradun: Bishen Singh Mahendra Pal Singh, p 868.

Gaur RD. 1999. *Flora of the District Garhwal Northwest Himalaya (with ethnobotanical notes)*. Transmedia, Srinagar Garhwal, India, p 811.

Hartmann HT, Kester DE, Davis FT. 1990. *Plant Propagation: Principles and Practices* (5th ed). New Jersey: Prentice-Hall, Englewood Cliffs, p647.

Hendry GAF, Price AH. 1993. Stress indicators: chlorophylls and carotenoids. In: Hendry, GAF, Grime JP (eds), *Methods in Comparative Plant Ecology*. London: Chapman & Hall, p148−152.

Hooker JD. 1894 (vol. 5th). *The Flora of British India*. Dehradun: Bishen Singh Mahendra Pal Singh, p 791.

Huxley AJ, Griffiths M, Levy M. 1992. *The New Royal Horticultural Society Dictionary of Gardening*. London: Macmillan Reference Limited, p33−34.

Khan AA, Heit CE. 1969. Selective effects of Hormones on nucleic acid metabolism during germination of pear embryos. *Biochemistry Journal*, **113**: 707−712.

Mamo N, Mihretu M, Fekadu M, Tigabu M, Teketay D. 2006. Variation in seed and germination characteristics among *Juneperus procera* populations in Ethiopia. *Forest Ecology and Management*, **225:** 320−327.

Matthews V. 1994. *The New Plantsman* (vol 1). London: Royal Horticultural Society, ISBN 1352−4186.

McIntyre GI, Cessna AJ, Hsiao AI. 1996. Seed dormancy in *Avena fatua*: interacting effects of nitrate, water and seed coat injury. *Physiologia Plantarum*, **97**: 291−302.

Nikolaeva MG. 1969. *Physiology of deep dormancy in Seeds*. Leningrad. lsdakel'stvo Nauka. Isr. Prog. Sci. Tranal., Jerusalem (Translated from Russian by Z. Shapiro, National Science Foundation, Washington D.C.), p220.

Pandey H, Nandi SK, Nadeem M, Palni LMS. 2000. Chemical stimulation of seed germination in *Aconitum heterophyllum* Wall. and *A. balfourii* Stapf.: Important Himalayan species of medicinal value. *Seed Science Technol*, **28**: 39−48

Pearson MC, Rogers JA. 1962. *Hippophae rhamnoides* L. *Journal of Ecology*, **50**: 501−513.

Rawat BS, Sharma CM, Ghildiyal SK. 2006. Improvement of Seed germination in three important conifer species by Glibberellic acid (GA_3). *Lyonia*, **11** (2): 23−30.

Sankhyan HP, Sehgal RN, Bhrot NP. 2005. Standardization of presowing treatments for different seabuckthorn species in cold deserts of Himachal Pradesh. *Indian Forester*, **131**(7): 931−938.

Siril EA, Dhar U, Dhyani PP. 1998. Seed germination of Chinese Tallow tree (*Sapium sebiferum*). *Forests, Farm and Community Tree Research Reports*, **3**: 55−58.

Snedecor GW, Cochran WG. 1967. *Statistical Methods (*6th ed). Ames: Iowa State University Press, p421−432.

Stokes P. 1965. Temperature and seed dormancy. *Encyclopaedia of Plant Physiology*, **15**: 746−803.

Tewari A, Dhar U. 1996. An investigation on seed germination of Indian Butter tree *Aisandra butyracea*. *Seed Science Technology*, **24**: 211−218.

Wilkinson L. 1986. SYSTAT: The system for statistics. Evanston, Illinois: SYSTAT Inc., p660.